

Page 14, line 16, change "Description of the Figure:" to --BRIEF DESCRIPTION OF THE DRAWINGS--;

between lines 23 and 24, insert the following heading:

--DESCRIPTION OF THE PREFERRED EMBODIMENTS--.

IN THE CLAIMS:

Replace all the claims now in the application with the following new claims:

Sub E
--44. Monocyte-derived antigen-presenting cells (MD-APCs) which have phagocytic capacity and which have capacity for MHC class I (MHC-I) and MHC class II (MHC-II) antigen presentation.

--45. The monocyte-derived antigen-presenting cells of claim 44, wherein said phagocytic capacity is exhibited by a high rate of phagocytic uptake of formalin-fixed yeast.

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--46. The monocyte-derived antigen-presenting cells of claim 44, wherein said capacity for antigen presentation is evidenced by the property of stimulating the proliferation of allogenic lymphocytes as measured in an allogenic primary mixed lymphocyte reaction (MLR) test.

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--47. The monocyte-derived antigen-presenting cells of claim 44, wherein said phagocytic capacity is evidenced by an uptake of formalin-fixed yeast after culturing macrophages for 2 hours, adding yeast in 1/10 macrophages/yeast ratio and incubating at 37°C, 5% CO₂ atmosphere for 2-3 hours fixing by

the May-Grünwald-Giemsa (MGG) staining, and the percentage of phagocytic The monocyte-derived antigen-presenting cells being quantified by microscopic analysis.

--48. The monocyte-derived antigen-presenting cells of claim 44, wherein said monocyte-derived antigen-presenting cells present on their surface antigen MHC-II with a mean intensity of about 100 to about 400.

--49. The monocyte-derived antigen-presenting cells of claim 44, wherein said monocyte-derived antigen-presenting cells are substantially devoid of surface antigen CD83.

--50. The monocyte-derived antigen-presenting cells of claim 44, wherein said monocyte-derived antigen-presenting cells present adherent properties as determined by ^{MD APCs} ~~macrophages~~ cultured for 2 hours in culture medium of one of I.M.D.M. and R.P.M.I. on plastic flasks and the percentage of adherent cells is quantified by microscopic analysis.

--51. The monocyte-derived antigen-presenting cells of claim 44, with the property of stimulating the proliferation of allogenic lymphocytes as determined in an allogenic primary mixed lymphocytes reaction (MLR) test carried out by adding different numbers of MD-APCs to purified allogenic T cells.

--52. The monocyte-derived antigen-presenting cells of claim 47, wherein said monocyte-derived antigen-presenting

cells present on their surface antigen MHC-II with a mean intensity of about 100 to about 400.

--53. The monocyte-derived antigen-presenting cells of claim 47, wherein said monocyte-derived antigen-presenting cells are substantially devoid of surface antigen CD83 as determined by immunofluorescence staining and flow cytometry analysis.

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--54. The monocyte-derived antigen-presenting cells of claim 47, wherein said monocyte-derived antigen-presenting cells present adherent properties as determined by ^{MD APCs} ~~macrophages~~ cultured for 2 hours in a culture medium of one of I.M.D.M. and R.P.M.I..

Sub E3
--55. Monocyte-derived antigen-presenting cells (MD-APCs) which present the following properties:

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- (a) the presence on the MD-APC cell surface of surface antigens CD80 and CD86;
 - (b) the presence on the MD-APC cell surface of surface antigen CD14, and
 - (c) a phagocytic capacity.

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--56. The monocyte-derived antigen-present cells of claim 55, wherein the mean intensities of CD80 and CD86 surface antigens as determined by immunofluorescence staining and flow cytometry analysis are in the range of about 20 to about 200 ~~units~~.

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--57. The monocyte-derived antigen-presenting cells of claim 55, wherein the mean intensity of CD14 surface antigens as determined by immunofluorescence staining and flow cytometry analysis are in the range of about 5 to about 200 units.

--58. The monocyte-derived antigen-presenting cells of claim 55, wherein said phagocytic capacity is exhibited by a high rate of phagocytic uptake of formalin-fixed yeast.

--59. The monocyte-derived antigen-presenting cells of claim 55, further comprising the property of having present on their surface antigen CD40 and mannose receptor with a mean intensity of 50 to about 500.

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--60. The monocyte-derived antigen-presenting cells of claim 59, wherein said monocyte-derived antigen-presenting cells are substantially devoid of the surface antigens CD1a and CD1c.

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--61. The monocyte-derived antigen-presenting cells of claim 55, wherein said phagocytic capacity is evidenced by an uptake of formalin-fixed yeast after culturing macrophages for 2 hours, adding yeast in 1/10 macrophages/yeast ratio and incubating at 37°C, 5% CO₂ atmosphere for 2-3 hours fixing by the May-Grünwald-Giemsa (MGG) staining, and the percentage of phagocytic MD-APCs being quantified by microscopic analysis.

--62. The monocyte-derived antigen-presenting cells (MD-APCs) which present on their surface:

antigen CD14 and CD64 with a mean intensity of about 20 to about 200,

antigen CD80 and CD86 with a mean intensity of about 20 to about 200,

antigen CD40 and mannose receptor with a mean intensity of 50 to 500,

wherein said monocyte-derived antigen-presenting cells are substantially devoid of the surface antigens CD1a and CD1c,

the presence and mean intensities of CD14, CD64, CD80, CD86 being determined by immunofluorescence staining and flow cytometry analysis,

said MD-APCs present a phagocytic capacity as determined by an uptake of formalin-fixed yeast, after culturing macrophages for 2 hours, adding yeast in 1/10 macrophages/yeast ratio and incubating at 37°C, 5% CO₂ atmosphere for 2-3 hours fixing by the May-Grünwald-Giemsa (MGG) staining, and the percentage of phagocytic The monocyte-derived antigen-presenting cells being quantified by microscopic analysis, and

said MD-APCs present the property of stimulating the proliferation of allogenic lymphocytes as determined by an allogenic primary mixed lymphocytes reaction (MLR) carried out in 96-well microtiter plates by adding different numbers of The monocyte-derived antigen-presenting cells to allogenic T cells purified from buffy coats and after 5 days incubation at

37°C, and cell proliferation being assessed by a calorimetric method.

--63. The monocyte-derived antigen-presenting cells of claim 62, which present on their surface antigen MHC-II with a mean intensity of about 100 to about 400, as determined by immunofluorescence staining and flow cytometry analysis.

--64. The monocyte-derived antigen-presenting cells of claim 62, which are substantially devoid of surface antigen CD83 as determined by immunofluorescence staining and flow cytometry analysis.

--65. The monocyte-derived antigen-presenting cells of claim 62, which present adherent properties as determined by the ^{MD APCs}~~macrophages~~ being cultured for 2 hours in a culture medium of one of I.M.D.M. and R.P.M.I..

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--66. ^A~~The~~ monocyte-derived antigen-presenting cells (MD-APCs) culture wherein:

about 10% to about 50% of the monocyte-derived antigen-presenting cells present antigen CD14 on their surface,

about 10% to about 50% of the monocyte-derived antigen-presenting cells present antigen CD46 on their surface,

about 30% to about 100% of the monocyte-derived antigen-presenting cells present antigens CD80 and CD86 on their surface,

about 80% to about 100% of the monocyte-derived antigen-presenting cells present antigen MHC-II on their surface,

about 70% to about 100% of the monocyte-derived antigen-presenting cells present adherent properties,

about 30% to about 100% of the monocyte-derived antigen-presenting cells present a phagocytic property, and

each The monocyte-derived antigen-presenting cells having the above-mentioned properties being such that said properties are expressed of the intensities as specified in claim 62.

--67. Process for preparing a composition comprising The monocyte-derived antigen-presenting cells (MD-APCs) which present on their surface:

antigen CD14 and CD64 with a mean intensity of about 20 to about 200,

antigen CD80 and CD86 with a mean intensity of about 20 to about 200,

antigen CD40 and mannose receptor with a mean intensity of 50 to 500,

comprising the steps of preparing a culture of mononuclear cells in a culture medium containing chemical ligands of mononuclear cells and allowing differentiation into The monocyte-derived antigen-presenting cells.

--68. Process of claim 67, wherein the culture medium contains chemical ligands of mononuclear cells selected

from the group consisting a histamine, a cimetidine, and a H_2 antagonist without GM-CSF.

--69. Process of claim 67, wherein the culture medium contains chemical ligands of mononuclear cells, selected from the group consisting of a histamine, a cimetidine, and a H_2 antagonist, each in combination with GM-CSF.

--70. Process of claim 69, wherein histamine is present at a concentration of about 10^{-2} M to about 10^{-6} M, one of cimetidine and the H_2 antagonist is present at a concentration of about 10^{-4} M to about 10^{-9} M, and GM-CSF is present at a concentration of about 50 U/ml to about 2000 U/ml.

--71. Process of claim 70, wherein histamine is present at a concentration of about 10^{-4} M, one of cimetidine and the H_2 antagonist is present at a concentration of about 10^{-6} M, and GM-CSF is present at about 500 U/ml.

--72. Process of claim 67, further comprising the steps of:

isolating leukocytes from peripheral blood apheresis and removing platelets and anticoagulants from the apheresis product,

isolating mononuclear cell monocytes and lymphocytes from red cells and granulocytes in order to have less than 10% granulocytes and less than 5% red cells,

culturing the obtained mononuclear cells by placing them in an appropriate culture medium containing chemical

ligands of mononuclear cells for a time sufficient to obtain differentiated The monocyte-derived antigen-presenting cells and recovering one of the monocyte-derived antigen-presenting cells, the macrophages, and the lymphocytes.

--73. Process of claim 67, comprising the further step of adding to the culture one of the group consisting of:
crude antigens selected from the group consisting of autologous tumor membrane, killed tumoral cells, bacterial capsides, and viral homogenates cleared from nucleic acids, specific peptides against which an immune response is desired,

one of cDNA and genetic material linked to vectors to allow transfection of the monocyte-derived antigen-presenting cells with material coding for the relevant peptide or protein to be presented on the macrophage membrane and against which an immune response is desired, and

bispecific antibodies targeting on the one side, a surface antigen of the monocyte-derived antigen-presenting cells and, on the other side, a relevant antigen against which an immune response is desired.

--74. Method of claim 67, wherein chemical ligands are selected from the group consisting of detoxified lipid A, C3, and ligands of complement receptors, taxols, oxydoreductors to the TNF receptors and to vitamin D3 receptors.

--75. Pharmaceutical compositions containing as active substance monocyte-derived antigen-presenting cells of claim 62.

--76. Cellular vaccine compositions containing as active substance monocyte-derived antigen-presenting cells of claim 62.

--77. A medium comprising:

chemical ligands of mononuclear cells taken from the group consisting of histamine and cimetidine, each in combination or not with GM-CSF; and

elements necessary for the growth and differentiation of monocytes into the monocyte-derived antigen-presenting cells (MD-APCs) which present on their surface

antigen CD14 and CD64 with a mean intensity of about 20 to about 200,

antigen CD80 and CD86 with a mean intensity of about 20 to about 200,

antigen CD40 and mannose receptor with a mean intensity of 50 to 500,

wherein the monocyte-derived antigen-presenting cells are substantially devoid of the surface antigens CD1a and CD1c,

the presence and mean intensities of CD14, CD64, CD80, CD86 being determined by immunofluorescence staining and flow cytometry analysis,

the MD-APCs present a phagocytic capacity as determined by an uptake of formalin-fixed yeast, after culturing macrophages for 2 hours, adding yeast in 1/10 macrophages/yeast ratio and incubating at 37°C, 5% CO₂ atmosphere for 2-3 hours fixing by the May-Grünwald-Giemsa (MGG) staining, and the percentage of phagocytic The monocyte-derived antigen-presenting cells being quantified by microscopic analysis, and

the MD-APCs present the property of stimulating the proliferation of allogenic lymphocytes as determined by an allogenic primary mixed lymphocytes reaction (MLR) carried out in 96-well microtiter plates by adding different numbers of The monocyte-derived antigen-presenting cells to allogenic T cells purified from buffy coats and after 5 days incubation at 37°C, and cell proliferation being assessed by a calorimetric method.

--78. Cell processor kit comprising:

a recovery means for the recovery of lymphocytes and monocytes free of contaminants,

a preparing means for preparing a culture for one of recovered monocytes and lymphocytes and comprising chemical ligands of mononuclear cells,

a transfecting means for transfection of cultured cells, and

a target means for targeting antigens to monocyte-derived antigen-presenting cells.

--79. Cell processor kit of claim 78, further comprising:

a means for recovering and centrifuging blood to obtain a leukocyte concentrate,

a means for separating lymphocytes and monocytes from other white cells and for eliminating contaminating red cells,

a culture medium for the monocyte-derived antigen-presenting cells with complements and chemical ligands of mononuclear cells,

means for the conservation of the monocyte-derived antigen-presenting cells, and

buffer and wash solution.

--80. Products comprising the monocyte-derived antigen-presenting cells of claim 62 and lymphocytes as a combined preparation for one of simultaneous, separate and sequential use in cell therapy.

--81. Products of claim 80, wherein they comprise the monocyte-derived antigen-presenting cells and the lymphocytes in a ratio of 20% to 50% of the monocyte-derived antigen-presenting cells as expressed in cell number.

--82. Method for clinical treatment, comprising the administration of an effective amount of the monocyte-derived antigen-presenting cells as prepared by the process of claim 67.

--83. Method of claim 82, wherein the effective amount is an amount of about 10^8 to about 5×10^9 of the monocyte-derived antigen-presenting cells.

--84. Method of claim 82, for the treatment of any disorder, comprising the further step of administering lymphocytes in an amount of about 4×10^9 to about 10×10^9 lymphocytes.

--85. A method of preparing monocyte-derived antigen-presenting cells comprising the step of using chemical ligands of mononuclear cells, wherein the monocyte-derived antigen-presenting cells present on their surface:

antigen CD14 and CD64 with a mean intensity of about 20 to about 200,

antigen CD80 and CD86 with a mean intensity of about 20 to about 200,

antigen CD40 and mannose receptor with a mean intensity of 50 to 500,

the MD-APCs being substantially devoid of the surface antigens CD1a and CD1c,

the presence and mean intensities respectively of CD14, CD64, CD80, CD86 and the absence of CD1a and CD1c being determined by immunofluorescence staining and flow cytometry analysis,

the MD-APCs presenting a high phagocytic capacity as evaluated by an uptake of formalin-fixed yeast, after culturing the monocyte-derived antigen-presenting cells for 2 hours

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to select adherent cells, adding yeast in 1/10 MD-APCs/yeast ratio and incubating at 37°C, 5% CO₂ atmosphere for 2-3 hours fixing by the May-Grünwald-Giemsa (MGG) staining and the percentage of phagocytic the monocyte-derived antigen-presenting cells being quantified by microscopic analysis, and the MD-APCs presenting the property of stimulating the proliferation of allogenic lymphocytes as determined by an allogenic primary mixed lymphocytes reaction (MLR) carried out in 96-well microtiter plates by adding different numbers ranging from 2×10^3 to 2×10^5 in 100 μ l medium/well of the monocyte-derived antigen-presenting cells to 2×10^5 in 100 μ l medium/well of allogenic T cells purified from buffy coats and after 5 days incubation at 37°C, cell proliferation being assessed by a calorimetric method.--

Please charge the fee of \$195 for the five extra independent claims added herewith, and the fee of \$189 for the 21 extra claims of any type added herewith, to Deposit Account No. 25-0120.

R E M A R K S

The application has been amended so as to replace the claims previously in the case with claims redrafted as to form. Support for the recitations of the claims can be found in the originally filed claims as well as in the specification at least at page 3, beginning on line 10 and page 4, beginning on line 1.

The claims set forth particular features of the present invention concerning a previously unknown hybrid type of monocyte-derived antigen-presenting cell (MD-APC). The MD-APC of the present invention possesses some of the characteristics of the macrophage cell line, e.g., phagocytosis, but also other characteristics of dendritic cells, e.g., antigen-processing and antigen-presenting capability.

Until recent years it was uniformly taken as dogma that dendritic cells were utterly quite of phagocytosis, and that macrophages, although exhibiting a certain degree of antigen-presenting capacity, were not particularly effective in this regard. In sharp contrast to this, however, the present invention discloses MD-APCs that are capable of mounting both types of immunological response to foreign invaders while displaying a combination of surface antigens characteristic to some extent of macrophages and to some extent of dendritic cells.

Examination on the merits is respectfully requested. As applicant believes that the present application is in condition for allowance and an early indication of the same is also respectfully requested.

If the Examiner has any questions or requires clarification, the Examiner may contact the undersigned

CHOKRI et al. S.N. 09/194,053

attorney so that this application may continue to be expeditiously advanced.

Respectfully submitted,

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June 17, 1999